Seroprevalence of dengue in urban and rural settings in Kerala, India

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Dengue fever is a major public health concern in India and Kerala is one of the worst affected states in the country. Kanjirappally, Kottayam district, has been reported to be the epicentre of dengue in the state. In 2016, we carried out a post-epidemic seroprevalence survey in both urban and rural sites of this endemic foci to estimate the disease burden. A systematic sampling technique with a random start (household) was adopted in each study site. Overall, 938 individuals were enrolled in the survey, 470 in the urban and 468 in the rural sites belonging to 103 and 88 households respectively. Rapid diagnostic IgM/IgG test kit was used for the study. The overall dengue IgG seroprevalence observed was 51.28%. Urban sites had higher seroprevalence rate (59.8%) compared to rural sites (42.74%, $P < 0.01$). No gender difference was recorded in seroprevalence rates among the sites. Exposure was found to be more common among adults than children in both areas. Seroprevalence rate in children <10 years of age was found to be 15 times higher (44.61%) in urban sites, than that in the rural sites (3.03%). The present study indicates that more than half of the population is exposed to DENV (dengue virus) infection in this oldest focus of dengue in Kerala.

Keywords: *Aedes aegypti*, dengue, seroprevalence, urban and rural settings, vector control.

DENGUE fever is an important emerging tropical infectious disease and about half of world's population, living in 128 countries, is at risk (WHO Fact Sheet Dengue 2020; http://www.who.int/news-room/factsheets/detail/dengue-andsevere-dengue). This disease is caused by dengue virus (DENV), belonging to the genus *Flavivirus*, and is transmitted by the Aedes mosquitoes, viz. *Aedes aegypti* and *Aedes albopictus*. Four different serotypes of this virus are known, namely DENV-1, 2, 3 and 4, causing dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS)[1]. Dengue fever is a major public health concern in India. Different studies have estimated the annual burden of this disease in the country, ranging from 12,432 to 34 million cases. In recent years, incidence of dengue infection has shown an increasing trend in different regions of India[2] and in Kerala (Figure 1).

Kerala, with an area of about 1.18% of the land mass and 2.8% of the total population of India, contributes to more than 10% of the reported dengue cases in the country[3]. Although serological evidence of dengue infection was reported from the state as early as 1973 (ref. 4), the first major outbreak of the disease was recorded in 1997 in Kanjirappally taluk, Kottayam district, with 14 cases and 4 deaths. This was followed by a larger outbreak in 1998 with 67 cases and 13 deaths[5]. This region is considered as the epicentre of dengue in Kerala[6]. All four serotypes of dengue virus are found co-circulating here, and DENV-1, 2 and 3 are predominant[3].

Kerala is located in South India, bordered by the Western Ghats in the east and the Arabian Sea in the West. The climate is tropical with about 3000 mm rainfall annually, fed by southwest and northeast monsoons. The temperature ranges from 22°C to 31°C with relative humidity of 70–90%. About 28% of land area of the state is under forest cover. The tropical humid climate is conducive to the growth of vector mosquitoes *Ae. aegypti* and *Ae. albopictus*[7–9]. Kottayam district has extensive rubber plantations, and *Ae. albopictus* breeds profusely in the fixed or discarded rubber latex containers[3]. However, this

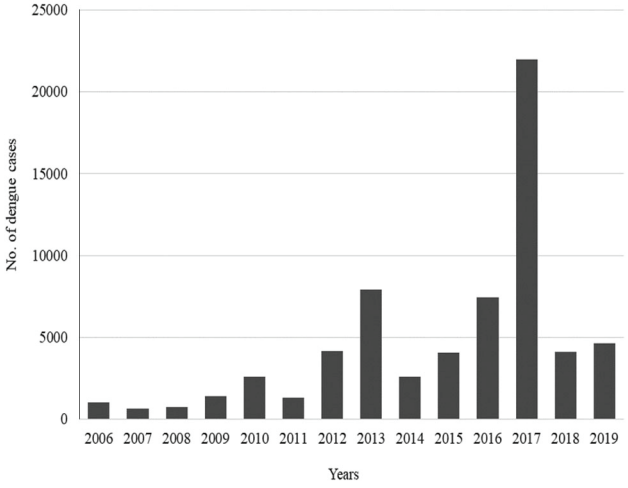

Figure 1. Reported number of dengue cases by the Integrated Disease Surveillance Programme in Kerala, India, during 2006–19.

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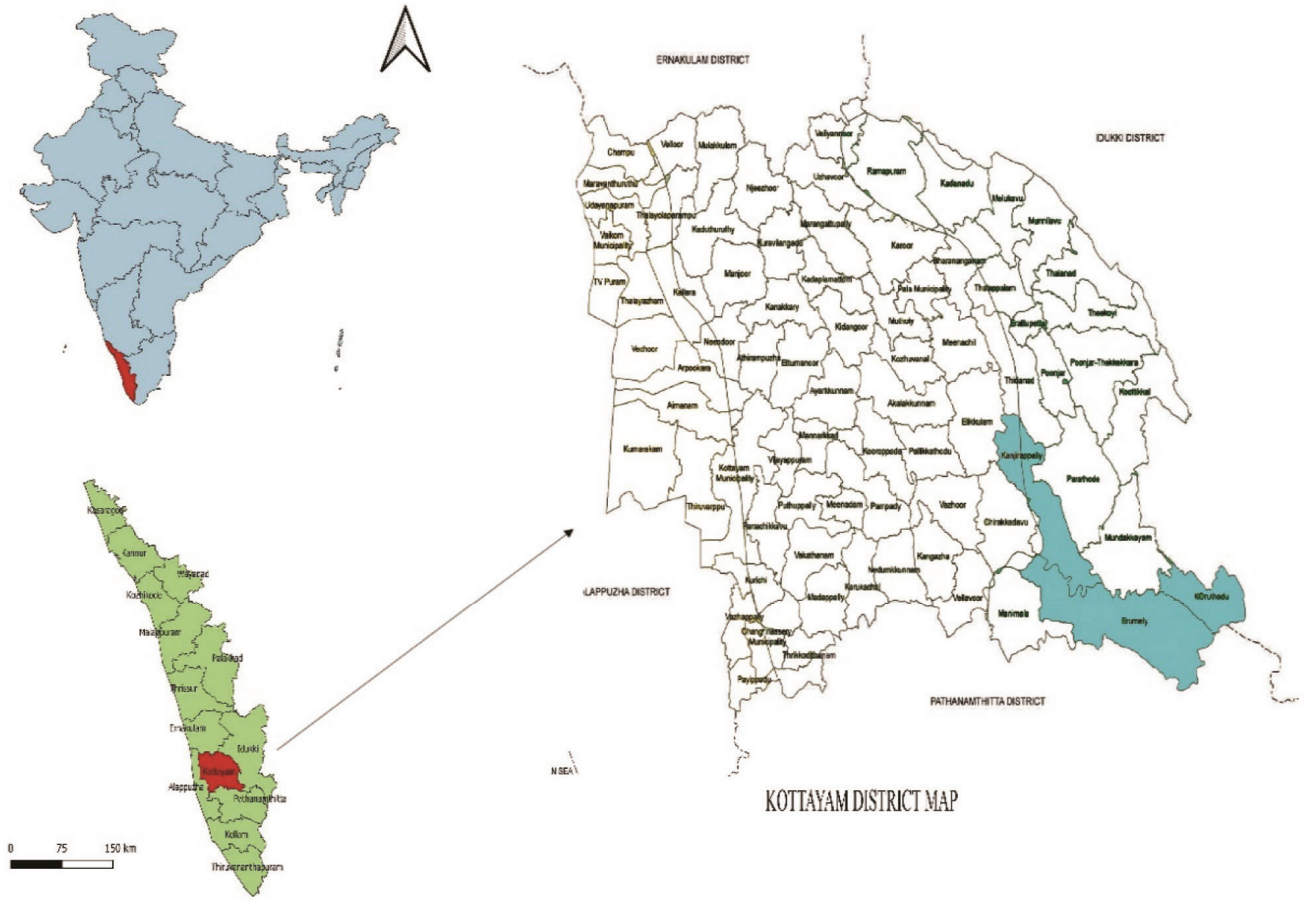

Figure 2. Map of Kerala showing the study areas: Kanjirappally (urban), and Erumeli and Koruthodu (rural).

species has not yet been incriminated as a vector of dengue in Kerala. *Ae. aegypti* is the established dengue vector and its breeding occurs mainly in the urban/semi-urban settlements[10,11]. The water-storage containers in urban conglomerations are the key breeding habitats of *Ae. aegypti*[12]. Unplanned rapid urbanization and climate change in recent years have aggravated the dengue crisis in the state.

Dengue infection is detected conventionally by immunological assays. Presence of dengue-specific immunoglobulin M (IgM) antibodies indicates current/recent infection, and immunoglobulin G (IgG) antibodies indicate past infection. Primary dengue virus infection is characterized by increase in IgM levels, 3 to 5 days after the onset of symptoms. IgM antibodies generally persist for 30 to 60 days[13]. IgG antibodies become detectable 10 to 14 days after the onset of symptoms and remain elevated lifelong[14]. During secondary dengue infection, IgM levels generally rise more slowly and reach lower levels than in primary infection, while IgG levels rise rapidly, 1 to 2 days after the onset of symptoms. Seroprevalence studies are especially useful for understanding the true burden of disease in the population in a locality.

Though dengue outbreaks have become a regular occurrence in Kerala, systematic data on infection status at population level are lacking and the problem is often underestimated. Therefore, a post-epidemic seroprevalence study was carried out in 2016, to estimate the magnitude of DENV infection in urban and rural areas of Kanjirappally taluk, and the results are presented here.

Materials and methods

Study area and sample collection

One urban area, Kanjirappally municipality (9°33′26.17″N, 76°47′21.96″E) and two rural areas, namely Koruthodu (9°28′33.6″N, 76°57′6.26″E) and Erumeli (9°26′4.56″N, 76°56′4.56″E) located in Kottayam district, worst affected by dengue, were selected as the study sites (Figure 2). The total population of the urban area was 4758 with 1030 households and in rural areas, it was 3785 with 883 households. These three study sites contributed to about 80.0% of DENV cases in the Kanjirappally taluk (VCRC Annual Report, 2013; http://vcrc.res.in/writereaddata/AR_2013.pdf).

Table 1. Dengue seroprevalence in the study sites

Study area	Population (*n*)	No. of participants			Dengue IgG positives (*n*)			Prevalence (%)		
		Male	Female	Total	Male	Female	Total	Male	Female	Total
Urban	4758	235	235	470	145	136	281	61.7	57.87	59.8
Rural	3785	233	235	468	106	94	200	45.49	32.34	42.74
Total	8543	468	470	938	251	230	481	53.63	48.93	51.28

Initially, a pre-test survey (2%) was conducted in the study sites to estimate the sample size. Also, 159 individuals residing in the study sites were randomly selected for the survey. Among them, 85 individuals were found to be reactive for dengue IgG. The seroprevalence rate was 53.24% in the urban site and 53.65% in rural sites. Based on this, sample size of 400 (9.7% of the population) in both urban and rural settings was estimated, assuming a seroprevalence rate of ~50% and a margin of error of 5% with 95% confidence level. Among the 938 individuals enrolled in the survey, 470 were in the urban site and 468 in the rural sites belonging to 103 and 88 households respectively.

A systematic sampling technique with a random start (household) was adopted in each study site. All the available individuals in the selected households at the time of study were enrolled and tested. Apart from immunological screening, demographic details of the study participants as well as clinical history of previous dengue fever episodes were recorded for each participant. The survey was conducted during the period February to September 2016.

Rapid detection test of dengue IgG/IgM

Different rapid diagnostic test (RDT) kits are available for detecting dengue infection[15]. In India, two commercially available rapid immunodiagnostic kits are being widely used for dengue diagnosis: PanBio (PanBio Diagnostics, Australia: Dengue Duo Cassette (Duo Cassette)) and SD Bioline (Standard Diagnostics, Republic of Korea: SD Bioline Dengue IgG/IgM). To evaluate the utility of these kits for the present study, known dengue-positive samples (n = 10; confirmed by RT-PCR and gene sequencing) were tested. While the SD Bioline kit detected all these cases to be seropositive, Pan Bio did not yield positive results for any of the samples. The failure in detection of positivity for the latter might be due to the variations in the strain circulating in the region, as described elsewhere[16]. From this preliminary observation, we selected SD Bioline IgG/IgM WB RDT kits for this study. Previous studies have also reported 90.4% (84.6–94.2) sensitivity and 88.9% (77.8–94.8) specificity for the SD Bioline kit[17–19]. Testing was done according to the instructions of the manufacturer. Briefly, by finger prick method, 10 µl of blood was taken from the patient and added into the sample well of the device, marked 'S', using a disposable dropper; the test result was interpreted in 15–20 min. The presence of only one pink band, within the result window, indicated negative result and the presence of two pink bands ('T' and 'C') indicated a positive result. The test was considered invalid if no control band (C) was observed.

Entomological collections

Apart from the serosurveys, fortnightly entomological analyses were conducted in two sentinel sites in each study site, to assess the vector population density. Both immature and adult collections were carried out following the standard procedures[20]. Indoor adult collections were made using mechanical aspirators, spending six man-hours (15 min/house for 24 houses) in an area. Outdoor collections were made using sweep-nets for three man-hours (30 sweeps/person/h). Immature surveys were carried out in an area of about 0.5 km^2 radius in plantation areas and domestic/peridomestic areas around the houses. All the pupae and immatures collected were brought to the laboratory and maintained for emergence, for species identification and detection of dengue viral infection.

Data analysis

GraphPad Prism 6.0 was used for graphical representation of the results. Statistical analysis was carried out using EPISTAT software version 16.0 (CDC, Atlanta, USA). Chi Square and Fisher's Exact tests were performed to compare the differences in seropositivity in relation to gender and different age groups in both urban and rural study areas. P-value <0.05 was considered statistically significant in all the tests.

Results

Seroprevalence of surveyed individuals

Among 938 individuals surveyed, 481 (51.28%) were found to be positive for DENV IgG; 281 (59.8%) in the urban site and 200 (42.74%) in the rural sites. The seroprevalence rate in the urban site was found to be significantly higher than that in the rural sites (P = 0.0001; Table 1).

Table 2. Seroprevalence rates in different age groups in urban and rural study sites

Age group (yrs)	Urban					Rural				
	Total no. of samples (*n*)	IgM *n* (%)	IgG only *n* (%)	IgG + IgM *n* (%)	Total IgG seropositives *n* (%)	Total no. of samples (*n*)	IgM *n* (%)	IgG only *n* (%)	IgG + IgM *n* (%)	Total IgG seropositives *n* (%)
0–10	65	1 (1.53)	22 (33.85)	7 (10.7)	29 (44.61)	33	0 (0)	1 (3.03)	0 (0)	1 (3.03)
11–20	50	1 (2.0)	19 (38.0)	7 (14.0)	26 (52.0)	70	2 (2.86)	12 (17.14)	6 (8.57)	18 (25.71)
21–30	90	1 (1.11)	36 (40.0)	15 (16.7)	51 (56.66)	56	0 (0)	10 (17.86)	5 (8.93)	15 (26.78)
31–40	75	0 (0)	42 (56.0)	8 (10.7)	50 (66.66)	92	1 (1.08)	31 (33.7)	12 (13.04)	43 (46.74)
41–50	78	2 (2.56)	40 (51.3)	13 (16.7)	53 (67.95)	71	1 (1.4)	32 (45.07)	8 (11.27)	40 (56.34)
>50	112	0 (0)	61 (54.46)	11 (9.82)	72 (64.28)	146	2 (1.37)	59 (40.41)	24 (16.44)	83 (56.85)
Total	470	5 (1.06)	220 (46.8)	61 (12.98)	281 (59.8)	468	6 (1.3)	145 (30.98)	55 (11.75)	200 (42.735)

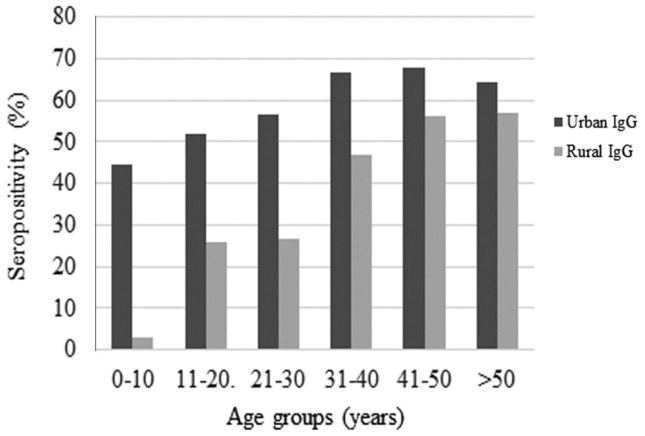

Figure 3. Age-specific dengue immunoglobulin G (IgG) prevalence in urban and rural study sites of Kanjirappally during 2016.

Sex-wise and age-wise seroprevalence rates

Male to female ratio in the study sites was found to be 1.02. No significant difference in seroprevalence rates was observed between males and females either in the urban ($P = 0.45$) or rural ($P = 0.26$) sites. A gradual and steady increase in seroprevalence status with age was noted in the urban as well as rural study sites (Table 2). Among the different age groups, seroprevalence was found to be uniformly higher in the urban site compared to that in the rural sites (Figure 3). Exposure was found to be more common among adults than children. In the urban site, seropositivity ranged from 44.61% in the 0–10 years age group to 64.28% in those >50 years of age. Seroprevalence rate in children <10 years of age was found to be 15 times higher (44.61%) in the urban site than in the rural sites (3.03%). The maximum seroprevalence was noted in the urban site in those belonging to >30 years of age (66.04%), while the corresponding figure was 53.72% in the rural sites ($P = 0.0027$; Table 2). Also, 6.8% of people in the urban site and 7.48% in the rural sites reported to have had a history of dengue fever. Overall, IgM seropositivity rate was 13.54% and was comparable between the urban and rural sites (14.04% versus 13.03%). In the urban site 12.8% of people were positive for both IgG and IgM, whereas 11.96% were seropositive for both in the rural sites.

Vector prevalence

Ae. aegypti was found to be the predominant vector species in the urban site with 44.5% prevalence and *Ae. albopictus* in the rural sites with 40.08% prevalence. *Ae. aegypti* was not present among the sampled mosquitoes from the rural sites. Key breeding habitats for *Ae. aegypti* in urban areas were domestic and peri-domestic water-storage containers. Small-scale rubber plantation areas were found interspersed in the rural study villages. Also, 29.9% of breeding of *Ae. albopictus* was contributed by the latex collection containers (either unused or discarded) associated with these rubber plantations and 27.3% by the peri-domestic discarded containers.

Discussion

Seroprevalence in urban and rural areas

Population-level dengue IgG prevalence in an endemic area reflects the cumulative effect of past dengue infections. In the present study, an overall seropositivity rate of 51.28% was observed in Kanjirappally taluk. Relatively higher rate was observed in the urban site in comparison to the rural sites (59.8% versus 42.74%). The overall seroprevalence rate observed in this study was similar to that reported in a countrywide survey conducted in 2017 (ref. 21). In that study, survey was conducted in 240 clusters selected from 60 districts of 15 Indian states from five geographical regions and an overall seroprevalence rate of 48.7% was recorded. The study also reported considerable heterogeneity in the burden of dengue infection in different geographical regions with high transmission observed in northern (60.3%), western (62.3%) and southern regions (76.9%). In another study performed ten years earlier, the urban areas of Delhi recorded a seropositivity of 45.13% (ref. 22). An IgG positivity of 58.5%

was reported in urban and 41.2% in rural areas of Pune, Maharashtra, India[9]. A seroprevalence of 65.42% was reported from Zhejiang Province, China, in 2012, following a dengue outbreak in 2009 (ref. 23).

Sex-wise and age-wise seroprevalence rates

No significant difference in gender-wise seroprevalence rates was observed, either in urban or rural sites. Both sexes have equal chances of exposure to dengue vectors. Seroprevalence of dengue IgG antibodies was found to be similar between males (73.81%) and females (60.00%) in serosurveys conducted elsewhere[23]. Seroprevalence increased significantly with increase in age in both urban and rural areas, which could be attributed to the age-related cumulative exposure to dengue virus. It is possible that older people are more likely to have been exposed to arboviruses throughout their lifetime. This pattern is evident in similar studies conducted by other researchers in India and elsewhere[23–27]. A seropositivity of 28.3% among children aged 5–8 years to 41.0% among children aged 9–17 years and 56.2% among those between 18 and 45 years of age was observed in a countrywide cross-sectional seroprevalence study of dengue in India[21]. A notable finding in the present study is that in urban areas, the 0–10 years age group showed a higher seroprevalence rate (44.62%) in comparison to that of rural area (3.03%). Previously, 59.6% dengue seroprevalence in children and 55% in adults belonging to the age group >40 years was reported in a serosurvey among blood donors in Singapore[28]. A similar trend was noticed in the present study too. A seroprevalence of 49.9% was recorded in the age group >10 years in a rural village, while it was >70% in an urbanized village during a serosurvey conducted in Pune[27]. In the present study, the IgM seropositivity was only 13.54%. The low IgM rates could be attributed to the study being undertaken during post-dengue outbreak, rather than during the outbreak period.

Vector prevalence

A probable reason for the differences in seroprevalence rate between urban and rural sites in this study could be attributed to the vector-specific characteristics in these two different settings. *Ae. aegypti* was found to be the predominant vector species (44.48%) in the urban site, followed by *Ae. albopictus* (40.2%). In the rural sites, *Ae. albopictus* (40.08%) was the major species, breeding profusely in rubber latex collection containers (either unused or discarded) associated with the rubber plantations, followed by peri-domestic discarded containers. *Ae. aegypti* was not present among the mosquitoes collected during the study period. We consider that the paucity of *Ae. aegypti* (which has been reported to be a more efficient vector species for dengue) in rural sites might be one of the reasons for less prevalence of dengue infection in rural areas, apart from the lack of other urban-specific factors. Screening of *Ae. albopictus* sampled in the study area for dengue infection did not yield any DENV-positive specimens by RT-PCR during two consecutive years. The results of the entomological studies suggest that the foci of transmission in the taluk could be the Kanjirappally urban area, where *Ae. aegypti* population was found to be comparatively higher than the villages. In addition, several pools collected during the peak transmission season were found infected with dengue virus in our ongoing studies. Unlike other regions of India, maximum number of cases of dengue in Kerala was reported during the pre-monsoon (May–July), owing to intermittent summer rainfall that is abundant in the state[3,12,29]. During summer season, *Ae. aegypti* breeds in water-storage containers in households in urban areas. The association of dengue prevalence with household water storage has also been reported elsewhere[29]. High dengue seroprevalence in urban areas of Kanjirappally taluk might be due to urbanization, higher population density and higher degree of exposure to infective bites of *Ae. aegypti*. Similar observations were made during seroprevalence studies carried out in forest fringe areas of Peninsular Malaysia[30].

Measures for vector control and dengue management

Integrated vector management strategy, including source reduction, proper solid-waste disposal, improved water-storage practices, and self-protection measures like mosquito nets and repellent use need to be advocated with active community participation, in order to contain the regular dengue outbreaks in the study region. IEC (information, education and communication) campaigns need to include community health workers, students and self-help groups like Anganwadi and Kudumbashree volunteers. Community education campaigns should especially focus on proper storage of water in containers, avoiding prolonged storage in open containers for more than a week. As latex-collection containers are the main source of breeding habitats in rubber plantation areas, workers and residents in these areas need to be made aware of the situation. Biocontrol with small larvivorous fishes such as *Poecilia reticulata*[31] or sturdier *Betta splendens*[32] could be explored in large and perennial water-storage containers.

Conclusion

This seroprevalence study of DENV infection in a hyper-endemic area in Kerala shows a high magnitude of dengue infection in the state. There is a need for strengthening the vector control activities in the region, with active community participation.

Ethical approval: The Institutional Ethics Committee of ICMR-Vector Control Research Centre, Puducherry (ECR 681/INST/PY/2014) approved this study. The participants were informed about the study and written informed consent was obtained from them.

Conflict of interest: The authors declare no competing interests.

1. Wang, W. H., Urbina, A. N., Chang, M. R., Assavalapsakul, W., Lu, P. L., Chen, Y. H. and Wang, S. F., Dengue hemorrhagic fever – a systemic literature review of current perspectives on pathogenesis, prevention and control. *J. Microbiol. Immunol. Infect.*, 2020, S1684-1182(20)300670; doi:10.1016/j.jmii.2020.03.007.
2. Ganeshkumar, P. *et al*., Dengue infection in India: a systematic review and meta-analysis. *PLoS Negl. Trop. Dis*., 2018, **12**(7), e0006618.
3. Kumar, N. P., Jayakumar, P. R., George, K., Kamaraj, T., Krishnamoorthy, K., Sabesan, S. and Jambulingam, P. J., Genetic characterization of dengue viruses prevalent in Kerala State. *Indian J. Med. Microbiol*., 2013, **62**, 545–552.
4. Banerjee, K. and Desai, P. K., Survey of arbovirus antibodies in South India. *Indian J. Med. Res*., 1973, **61**, 344–351.
5. Tyagi, B. K., Hiriyan, J., Samuel, P., Tiwari, S. C. and Paramasivan, R., Dengue in Kerala: a critical review. *ICMR Bull*., 2006, **36**(4–5), 13–22.
6. Kalra, N. L. and Prasittisuk, C., Sporadic prevalence of DF/DHF in the Nilgiri and Cardamom hills of Western Ghats in South India: is it a seeding from sylvatic dengue cycle – a hypothesis. *Dengue Bull*., 2004, **28**, 44–50.
7. Gubler, D. J., Dengue and Dengue haemorrhagic fever. *Clin. Microbiol. Rev*., 1998, **11**(3), 480–496.
8. Kalra, N. L., Kaul, S. M. and Rastogi, R. M., Prevalence of *Aedes aegypti* and *Aedes albopictus* – vectors of dengue and dengue haemorrhagic fever in north, north-east and central India. *Dengue Bull*., 1997, **21**, 84–92.
9. Cecilia, D., Current status of dengue and chikungunya in India. WHO South East. *Asian J. Pub. Health*, 2014, **3**(1), 22–26.
10. Kumar, N. P., Joseph, R., Kamaraj, T. and Jambulingam, P., A226V mutation in virus during the 2007 chikungunya outbreak in Kerala. *Indian J. Gen. Virol*., 2008, **89**, 1945–1948.
11. Kumar, N. P. *et al*., Chikungunya virus outbreak in Kerala, India, 2007: a seroprevalence study. *Mem. Inst. Oswaldo Cruz*, 2011, **106**, 912–916.
12. Kumar, N. P., Anish, T. S., Mathew, J., Valaparambil, A. T. T., Abidha, J. M., Ajithlal, P. M. and Jambulingam, P., Genotype shift of dengue virus (DENV1) during the 2017 outbreak of dengue fever in Thiruvananthapuram Kerala, India. *Ind. J. Exp. Biol*., 2019, **57**, 961–966.
13. Lam, S. K., Rapid dengue diagnosis and interpretation. *Malays. J. Pathol*., 1993, **15**, 9–12.
14. Gubler, D. J., Serological diagnosis of dengue/dengue haemorrhagic fever. *Dengue Bull*., 1996, **20**, 20–23.
15. Craig, A. T. *et al*., Enhanced surveillance during a public health emergency in a resource-limited setting: experience from a large dengue outbreak in Solomon Islands, 2016–17. *PLoS ONE*, 2018, **13**(6), e0198487.
16. Pal, S. *et al*., Evaluation of Dengue NS1 antigen rapid tests and ELISA kits using clinical samples. *PLoS ONE*, 2014, **9**(11), 1.
17. Vickers, I. E., Harvey, K. M., Brown, M. G., Nelson, K., Du Casse, M. B. and Lindo, J. F., The performance of the SD BIOLINE Dengue DUO® rapid immunochromatographic test kit for the detection of NS1 antigen, IgM and Ig antibodies during a dengue type 1 epidemic in Jamaica. *J. Biomed. Sci*., 2015, **22**, 55.
18. Blacksell, S. D. *et al*., The comparative accuracy of 8 commercial rapid immunochromatographic assays for the diagnosis of acute dengue virus infection. *Clin. Infect. Dis.*, 2006, **42**(8), 1127–1134.
19. Blacksell, S. D., Commercial dengue rapid diagnostic tests for point-of-care application: recent evaluations and future needs? *J. Biomed. Biotechnol*., 2012, **12**, 151967; doi:10.1155/2012/151967.
20. Focks, D. A., A review of entomological sampling methods and indicators for dengue vectors. World Health Organization, Geneva, 2003; Document WHO/TDR/IDE/Den/03.1.
21. Murhekar, M. V. *et al*., Burden of dengue infection in India, 2017: a cross-sectional population based serosurvey. *Lancet Glob. Health*, 2019, **7**(8), e1065–e1073.
22. Gupta, N., Srivastava, S., Jain, A. and Chaturvedi, U. C., Dengue in India. *Indian J. Med. Res*., 2012, **136**(3), 373–390.
23. Luo, S., Cui, W., Li, C., Ling, F., Fu, T., Liu, Q., Ren, J. and Sun, J., Seroprevalence of dengue IgG antibodies in symptomatic and asymptomatic individuals three years after an outbreak in Zhejiang Province, China. *BMC Infect. Dis*., 2018, **18**, 92.
24. Pavía-Ruz, N. *et al*., Seroprevalence of dengue antibodies in three urban settings in Yucatan, Mexico. *Am. J. Trop. Med. Hyg*., 2018, **98**(4), 1202–1208.
25. Fritzell, C., Rousset, D., Adde, A., Kazanji, M., Van Kerkhove, M. D. and Flamand, C., Current challenges and implications for dengue, chikungunya and Zika seroprevalence studies worldwide: A scoping review. *PLoS Negl. Trop. Dis*., 2018, **12**(7), e0006533.
26. Dhanoa, A. *et al*., Seroprevalence of dengue among healthy adults in a rural community in Southern Malaysia: a pilot study. *Infect. Dis. Poverty*, 2018, **7**, 1; https://doi.org/10.1186/s40249-017-0384-1
27. Shah, P. S. *et al*., Seroprevalence of dengue in a rural and an urbanized village: a pilot study from rural western India. *J. Vector-Borne Dis*., 2017, **54**(2), 172–176.
28. Low, S. L., Lam, S., Wong, W. Y., Teo, D., Ng, L. C. and Tan, L. K., Dengue seroprevalence of healthy adults in Singapore: serosurvey among blood donors. *Am. J. Trop. Med. Hyg*., 2015, **93**(1), 40–45.
29. Garg, S., Chakravarti, A., Singh, R., Masthi, N. R., Goyal, R. C., Jammy, G. R. and DNG10 study group. Dengue serotype-specific seroprevalence among 5 to 10-year-old children in India: a community-based cross-sectional study. *Int. J. Infect. Dis.*, 2017, **54**, 25–30.
30. Juraina, A. J. *et al*., Possible factors influencing the seroprevalence of dengue among residents of the forest fringe areas of Peninsular Malaysia. *J. Trop. Med*., Article ID 1019238, 2020, 10; https://doi.org/10.1155/2020/1019238
31. Seng, C. M., Setha, T., Nealon, J., Socheat, D., Chantha, N. and Nathan, M. B., Community-based use of the larvivorous fish *Poecilia reticulata* to control the dengue vector *Aedes aegypti* in domestic water storage containers in rural Cambodia. *J. Vec. Ecol*., 2008, **33**(1), 139–144.
32. Cavalcanti, L. P., Coelho, I. C., Vilar, D. C., Holanda, S. G., Escóssia, K. N. and Souza-Santos, R., Clinical and epidemiological characterization of dengue hemorrhagic fever cases in northeastern Brazil. *Rev. Soc. Bras. Med. Trop*., 2010, **43**(4), 355–358.

ACKNOWLEDGEMENTS. This study was funded under Intramural Project ID: IM1303 of the Indian Council of Medical Research – Vector Control Research Centre (VCRC), Puducherry. We thank P. Jambulingam (former Director, VCRC), S. Sabesan and S. Subramaniam (Senior Consultants, VCRC, Puducherry) for guidance, and K. S. Snehalatha, Ambilikumar, Esther Nirmala Mary, M. Balamurugan, P. Shantha Kumar and K. P. Amju, VCRC Field Station, Kottayam, Kerala for technical assistance.

Received 21 December 2020; revised accepted 28 May 2021

doi: 10.18520/cs/v121/i2/233-238